APPLICATION OF FLUORESCENCE MICROSCOPY TO EVALUATE THE VIABILITY OF BONE MARROW CELLS DURING THEIR PRESERVATION

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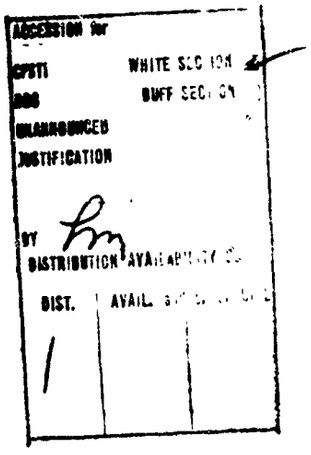

APPLICATION OF FLUORESCENCE MICROSCOPY TO EVALUATE THE VIABILITY OF BONE MARROW CELLS DURING THEIR PRESERVATION

[Following is the translation of an article by V. G. Mikhaylov, Uzbek Scientific-Research Institute of Hematology and Blood Transfusion, Tashkent, published in the Russian-language periodical Izvestiya AN SSSR, seriya biologicheskaya (Bulletin of the USSR Academy of Sciences, Biology Series), 1965, No 6, pages 932--934. It was submitted on 24 Nov 1964. Translation performed by Sp// Charles T. Ostertag, Jr.]

In connection with the successful transplantation of bone marrow during a number of illnesses, an intensification has begun in the development of methods for its preservation after it has been taken from donors, patients and corpses. Preservation media have been proposed for the storage of bone marrow at 4--6° up to 10 days (Fedotenkov et al., 1963, Mikhaylov, Kalugina, 1964; Tsegoreli, Tungareli, 1964).

The most reliable method for verifying the effectiveness of the preservation solution is a verification of the capability of the preserved bone marrow to protect an animal from lethal irradiation. However, this method is very laborious and requires lengthy observation on a great number of irradiated animals, Determination of the viability of preserved bone marrow cells with the help of the method of tissue culture is also quite complex, and in order to obtain results it is necessary that observations be carried out for several days.

The laboriousness of the existing methods for determining the viability of bone marrow cells complicates the development of new preservation solutions for the storage of bone marrow, since it is necessary to make an investigation of the effect of the components of these preservatives on the cells and their various concentrations and combinations.

We made use of fluorescence microscopy for judging the viability of bone marrow cells during storage. The basis was the data of Strugger (1949) which stated that during staining with acridine orange fluorochrome injured cells took on a red fluorescence, while uninjured cells possess a green coloration. Though it was demonstrated that the Strugger effect is far from always reproducible (for a critical review of the literature, see Bertalanffy, Bickis, 1956), and that pathologically changed and dead cells from blood and blood-producing organs in some cases can give off a green coloration (Kosenow, 1956; Zakrzhevskiy, Vasilyeva, 1963), we assumed that the use of any express method, even though it did not give completely accurate data, was very important for evaluating preservatives.

Our method of vital staining with acridine orange was exceptionally simple. We placed a drop from a suspension of bone marrow cells on a slide and immediately added to it a similar drop of acridine orange, prepared in a physiological solution in a dilution of 1:10,000. Both drops were mixed and covered with a cover slip. The preparation was investigated using a fluorescence microscope at magnifications of 40 x 10x and 90 x 7x.

As is known (Robbins, Marcus, 1963), the concentration of hydrogen ions in the medium exerts an influence on the nature of fluorescence of the cells. In our cases a phosphate buffer was included in the composition of all the solutions studied for the storage of bone marrow. This guaranteed the optimal physiological conditions for the storage of marrow elements (pH of the medium 7.3--7.4). Therefore, the conditions for the observation of fluorescence were uniform.

Fluorescence microscopic investigation revealed the following picture. Cells from the white series: The myeloblasts have a quite large nucleus, fluorescing green; the nuclei of the myelocytes are colored bright green and there are red granules in the cytoplasm; the nuclei of the neutrophils fluoresce green and in them the bright red fluorescing cytoplasmic granularity is strongly expressed. The green fluorescence of lymphocyte nuclei is more intensive than in th neutrophils and is characteristic. The general background of the cytoplasm of all the cells of the white series is a pale green.

Cells of the red series: Proerythroblasts and erythroblasts have a green coloration for the nuclei and a dim green coloration for the protoplasm.

When studying bone marrow cells during storage changes are observed in the fluorescence of both the nuclei and the cytoplasm. The color of fluorescence of the nuclei during this changes gradually. Initially they acquire a yellow-green, then a yellow, and finally a red fluorescence. At the same time pyknosis and nuclear fragmentation take place.

The fluorescence of the cytoplasm also changes in the direction of red tones. Changes in the nuclei and cytoplasm, detected during the first days of storage with the help of fluorescence microscopy, are not noted during a morphological investigation of smears of preserved bone marrow. To a certain degree the changes in the fluorescence of bone marrow cells characterize the viability, and consequently the capability of young cells for maturing after transplantation, though in comparison with the more reliable methods of investigation, pointed out above, fluorescence microscopy exhibited a higher number of viable elements.

Following fluorochroming with acridine orange it was established that during the process of storing preserved bone marrow a decrease is observed in the number of cells having a green fluorescence of the nucleus and protoplasm and an increase in the number of cells with a red fluorescence. Thus, during the storage of bone marrow in one of the preserving solutions the percentage of cells having a green fluorescence during the first days of preservation comprised 96.5%, third day - 84%, fifth - 79%, seventh - 76%, and tenth - 68%.

An investigation was made of the viability of bone marrow cells in various variations of preservative media, proposed by us jointly with G. N. Kalugina. Bone marrow from 8--10 donors or corpses was put up in each medium. During the process of storage dynamic fluorescence microscopic investigations

were made of the cells. When investigated by the tissue culture method such an operation required a great deal of time. Subsequently from all of the variations of preservative media we selected the one which produced the best results. We then confirmed the effectiveness of this solution with other, more reliable, but much more laborious, methods.

For the more prolonged, several months or even years, storage of bone marrow we apply the method of freezing and preservation of bone marrow at low and ultralow temperatures (-79 and -196°) (Lochte et al., 1959; Revis, 1961; Cavins et al., 1962; Fedotenkov et al., 1963).

For storing cells at this temperature a special method of freezing is used, as well as various safety substances. The least traumatic method of freezing cells, developed at the present time, and also the selection of the most effective safety substances and their most optimum concentrations also give rise to the necessity of using an express method for evaluating the viability of bone marrow cells.

The method of fluorescence microscopy of bone marrow cells used by us made it possible to study their viability depending on the various methods of freezing and the action of the two safety substancess -- glycerin and dimethyl sulfoxide. Changes in the fluorescence of the cells were the same as during storage in liquid preservatives. The advantage of vital staining with acridine orange when studying the viability of bone marrow cells after thawing is still more apparent, since other laboratory methods do not produce satisfactory results in this case. This is explained by the fact that cells, having endured deep freezing, storage at ultralow temperatures and subsequent thawing become injurable easily and may be destroyed during the preparation of smears or other manipulations.

A comparative investigation on the freezing of bone marrow with glycerin and dimethyl sulfoxide showed the best preservation of the cells when using dimethyl sulfoxide as the safety substance and a very slow lowering of the temperature of the suspension of bone marrow with the preservative solution.

Conclusions

Fluorescence microscopy of bone marrow cells may serve as an express method for a comparative judgement of the viability of cellular elements during the preservation of bone marrow by the freezing method and in liquid media.

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